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The histone H3 Lys 27 demethylase KDM6B promotes migration and invasion of glioma cells partly by regulating the expression of *SNAI1*

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A R T I C L E I N F O

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A B S T R A C T

The histone demethylase KDM6B, also known as jumonji domain-containing protein 3 (JMJD3), is an epigenetic regulator which plays important roles in immune activation, tissue regeneration, cellular senescence and cancer metastasis. But, the role of KDM6B in glioma metastasis is poorly understood. In this study, we achieved transcriptional regulation of KDM6B in glioma cells using CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa). Our results showed that KDM6B promotes the proliferation, migration and invasion of human glioblastoma cells U87 and U251 using CCK8, scratch and transwell assays. Further results indicated that KDM6B increases the expression of SNAI1, a key factor of epithelial-mesenchymal transition (EMT). KDM6B catalyzes the demethylation of histone H3 Lys 27 trimethylation (H3K27me3) in the promoter of *SNAI1*, which is important for *SNAI1* upregulation. Taken together, these findings provide new insight into the mechanism by which KDM6B promotes glioma metastasis.

1. Introduction

Histones constitute the core component of the nucleosome, the basic unit of chromatin. Histone is not an inert structure, but has a wide range of regulatory functions (Li et al., 2007). Histone modifications regulate gene transcription by affecting chromatin compaction, DNA accessibility and recruitment of transcription factors (Bannister and Kouzarides, 2011). Histone methylation is an important and widespread type of histone modifications (Greer and Shi, 2012). Histone methylation is also a reversible process catalyzed by methyltransferases and demethylases (Kooistra and Helin K, 2012).

Lysine demethylase 6B (KDM6B), also known as jumonji domain-containing protein 3 (JMJD3), can catalyze demethylation of histone H3 Lys 27 trimethylation (H3K27me3) (Lan et al., 2007; Agger et al., 2007). Many studies have proved that KDM6B plays essential roles in development, immune regulation and sex determination (Burchfield et al., 2015; Ge C et al., 2018).

Abnormal expression or activity of KDM6B is closely related to cancer development (Arcipowski et al., 2016). KDM6B expression is upregulated in several cancers, such as Hodgkin's lymphoma (Anderton et al., 2011), melanoma (Park et al., 2016), and renal cell carcinoma (Shen et al., 2012). Our previous study showed that KDM6B is overexpressed in glioma cells (Sui et al., 2017), and another study indicated that higher levels of KDM6B are associated with the malignant grades of glioma (Perrigue et al., 2015). But the detailed molecular mechanism underlying KDM6B with glioma development remains to be further studied.

In the current study, we achieved the KDM6B expression regulation

Abbreviations: KDM6B, Lysine demethylase 6B; JMJD3, jumonji domain-containing protein 3; H3K27me3, histone H3 Lys 27 trimethylation; CRISPRi, CRISPR interference; CRISPRa, CRISPR activation; qPCR, quantitative polymerase chain reaction; ChIP, Chromatin immunoprecipitation; EMT, epithelial-mesenchymal transition

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with CRISPR interference (CRISPRi) and CRISPR activation(CRISPRa). We further observed that the levels of KDM6B expression are associated with cell proliferation, migration and invasion of gliomas. Our results also indicated that KDM6B can increase the expression of EMT marker SNAI1, and achieve the activity by decreasing the content of H3K27me3 in promoter region of *SNAI1*. Our findings revealed the role and potential mechanism of KDM6B in gliomas metastasis, which is important for diagnosis and therapy of malignant gliomas.

2. Material and methods

2.1. Cell culture

The human glioblastoma cell lines U87 and U251 were obtained from Cell Resource Center, Chinese Academy of Medical Sciences (Beijing, China). Both cells were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Grand Island, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, USA) and penicillin/streptomycin. Cells were cultured at 37 °C in a humidified atmosphere with 5% CO2.

2.2. CRISPR interference (CRISPRi)of KDM6B

For CRISPRi experiments, plasmid containing dead Cas9 (dCas9), Krüppel associated box (KRAB) domain and single guide RNA (sgRNA) was constructed by Syngentech (Beijing, China). The sequences of sgRNA were listed in Table 1.

For plasmid transfection, U87 and U251 cells were plated at a density of 1×10^5 per well of a 6-well plate. 1 μg plasmid was transfected by Lipofectamine 3000. At 48 h after transfection, the cells were collected for extraction of total RNA or protein. The knockdown efficiency was verified by qPCR and western blotting.

2.3. CRISPR activation(CRISPRa)of KDM6B

For CRISPRa experiments, plasmid containing dCas9, transcriptional activator VP16 and sgRNA was constructed by Syngentech. The sequences of sgRNA were also listed in Table 1. The procedure for plasmid transfection is similar to CRISPRi experiments. The over-expressed efficiency was detected by qPCR and western blotting.

2.4. RNA extraction and quantitative polymerase chain reaction (qPCR)

Total RNA was extracted with Trizol extraction kit (Invitrogen, Carlsbad, CA, USA). 1 μg RNA was used for each sample in a 20 μL reaction using a reverse transcription (RT) Kit (TaKaRa, Dalian, China). The qPCR reactions were prepared with a PCR kit (TaKaRa) and performed on LightCycler480 System (Roche, Foster City, CA, USA). The primers were synthesized by Sangon (Shanghai, China) and sequences were as follows.

KDM6B forward: 5′-CACCCCAGCAAACCATATTATGC-3’;
reverse: 3′- CACACAGCCATGCA GGG ATT -5’.
SNAI1 forward: 5′- AGATGAGCATTGGCAGCGAG -3’;
reverse: 3′- TCGGAAGCCTAACTACAGCGA -5’.
β-ACTIN forward: 5′-CCACTGGCATCGTGATGGACTCC-3’;
reverse: 5′-GCCGTGGTGGTGAAGCTGTAGC-3’.

Table 1
sgRNA sequence of CRISPRi or CRISPRa for *KDM6B*.

Type	sgRNA sequence(5′- 3′)
CRISPRa-sgRNA-1	TTCTCCTTAATGAGGGGGGC
CRISPRa-sgRNA-2	GATCGAGCCTGCTTCCCAAA
CRISPRi-sgRNA-1	GAGGAGTGTAGCAAGGAGTC
CRISPRi-sgRNA-2	GAGAAACCCCATATGTAATG
Control sgRNA	GACTGGCGGAGCGTGCTATC

All reactions were performed in triplicate. Relative mRNA expression was normalized to *β-ACTIN*.

2.5. Western blotting

Extraction of protein form cultured cells was performed with a radioimmunoprecipitation assay (RIPA) buffer containing a protease inhibitor cocktail and phenylmethylsulfonyl fluoride (PMSF). 50 μg protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were saturated with 5% milk in TBST (50 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20) and then incubated with primary antibodies at 4 °C overnight. The primary antibodies used in this study included rabbit polyclonal antibodies against KDM6B (1:1,000, Abcam, Shanghai, China), H3K27me3 (1:1000, Cell Signaling Technology, Danvers, MA, USA), SNAI1(1:1000, Cell Signaling Technology), H3(1:1000, Cell Signaling Technology) and β-ACTIN (1: 2,500, Abcam). The membranes were incubated with an HRP-conjugated goat anti-rabbit antibody (1:5,000, Cell Signaling Technology) for 2 h at room temperature and then exposed to the enhanced chemiluminescence substrate (Millipore, Rockford, IL, USA). Detection of the bands was performed using a fully automatic chemiluminescence image analysis system (TANON, Shanghai, China).

2.6. CCK8 assay

Cell proliferation was examined using the Transdetect Cell Counting Kit (Transgen, Beijing, China). U87 and U251 cells were grown in a 96-well plate for 24 h, and transfected with CRISPRa or CRISPRi plasmid. To measure the cell proliferation at 0, 24, 48, or 72 h after transfection, 10 μl of CCK-8 was added to each well and then the cells were cultured for 1 h. Absorbance was measured at a wavelength of 450 nm. Assays were repeated at least three times.

2.7. Scratch assay

The migration ability of U87 and U251 cells was analyzed with scratch assay. Both cells were seeded into 6-well plates (Corning, NY, USA), and transfected with CRISPRa or CRISPRi plasmid. A clear line was drawn with a sterile pipette tip after 24 h transfection. The cultured cells were imaged with light microscope at 0 h and 24 h. The relative cell migration rate was calculated according to the position of the line at 0 h and 24 h.

2.8. Transwell assay

The invasion ability was evaluated with transwell assay. U87 and U251 cells were transfected with CRISPRa or CRISPRi plasmid. After 24 h transfection, the cells were seeded in the upper chamber of the insert (BD Biosciences, FranklinLakes, NJ, USA) at a density of 2×10^4 cells in 200 μL of serum-free DMEM. The bottom of the insert contained DMEM with 10% FBS. After 24 h, the cells in the bottom of the insert were stained with crystal violet and analyzed under light microscope.

2.9. Chromatin immunoprecipitation (ChIP)

ChIP was performed with EZ-ChIP kit (No.17–371, Upstate, Millipore, USA). Briefly, transfected U87 and U251 cells were chemically cross-linked by 1% formaldehyde and the reaction was stop by adding 125 mM glycine. Cells were removed the medium and washed twice with cold PBS. Cells were collected and then sonicated at 4 °C. Lysates were clarified by centrifugation and supernatants were collected. The diluted supernatants were incubated with primary antibodies KDM6B or H3K27me3 overnight at 4 °C with rotation. For the negative control, rabbit IgG was added. Then, the protein G agarose was added to incubate for 2 h. Cross-links of protein-DNA complexes were reversed by adding 0.2 M NaCl overnight incubation at 65 °C and followed by protein digestion with proteinase K. Immunoprecipitated DNA was purified and used for gene-specific qPCR. The primers were synthesized by Sangon (Shanghai, China) and sequences were as follows. Forward: 5′-GGCACGGCCTAGCGAGT-3'; Reverse: 5′-AGTGGTC GAGGCACTGGG-3'. The amplified fragment is located in -70 —1 upstream of *SNAI1*.

2.10. Statistical analysis

Values are expressed as mean ± standard deviation (SD) from at least three independent experiments. The independent Student *t*-test was used to compare the difference between two groups. A *P* value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. The repression of KDM6B is achieved with CRISPRi

In order to investigate the role and mechanism of KDM6B, expression regulation of KDM6B should to be achieved firstly. CRISPRi can inhibit gene expression on the transcriptional level. Our results showed that the mRNA levels of *KDM6B* were reduced by 58 percent and 55 percent in glioma cells U87 with sgRNA-1 and sgRNA-2 respectively in CRISPRi assay, and by 52 percent and 61 percent in U251 ($P < 0.05$, Fig. 1A and C). The KDM6B protein was obviously decreased while the content of H3K27me3 increased (Fig. 1A and C). This means the gene knockdown of KDM6B is achieved.

3.2. The activation of KDM6B is achieved with CRSIPRa

We also performed the CRISPRa assay. The results indicated that the mRNA levels of KDM6B were increased by 3.8 folds and 4.3 folds in glioma cells U87 with sgRNA-1 and sgRNA-2 respectively in CRISPRa assay, and 4.5 folds and 3.3 folds in U251 ($P < 0.05$, Fig. 1B and D). The KDM6B protein was obviously increased while the content of H3K27me3 decreased (Fig. 1B and D). These results mean that the gene activation of KDM6B was achieved. The achievement of gene repression and activation is essential for further studies on KDM6B's biological function and mechanism.

3.3. KDM6B promotes cell proliferation of glioma cells

To determine the role of KDM6B on cell proliferation, we performed CCK-8 assay. The results showed that knockdown of KDM6B inhibited cell proliferation of U87 and U251, and overexpression promoted cell proliferation ($P < 0.05$, Fig. 2). This result is consistent with our previous study (Sui et al., 2017).

3.4. KDM6B promotes cell migration of glioma cells

The overexpression of KDM6B in glioblastoma cell lines U87 and U251 (Sui et al., 2017; Perrigue et al., 2015) suggested that KDM6B may have a metastasis promoting role. To test the hypothesis, we examined the effect of KDM6B knockdown and overexpression on the migration in U87 and U251 cells using scratch assay. Knockdown of KDM6B significantly inhibited cell migration in both cell lines ($P < 0.05$, Fig. 3). And, overexpression of KDM6B obviously enhanced migration ability ($P < 0.05$, Fig. 3).

3.5. KDM6B promotes cell invasion of glioma cells

We next investigated the effect of KDM6B on invasion abilities of U87 and U251 cells using in vitro transwell assay. Knockdown of KDM6B significantly suppressed cell invasive ability in both cell lines ($P < 0.05$, Fig. 4). And, KDM6B overexpression significantly promoted invasion of U87 and U251 cells ($P < 0.05$, Fig. 4).

3.6. KDM6B increases the expression of SNAI1 in glioma cells

Previous studies showed that KDM6B can promote the expression of SNAI1 in breast cancer cells (Ramadoss et al., 2012), but it is not clear in glioma. qPCR experiments indicated that KDM6B increased the

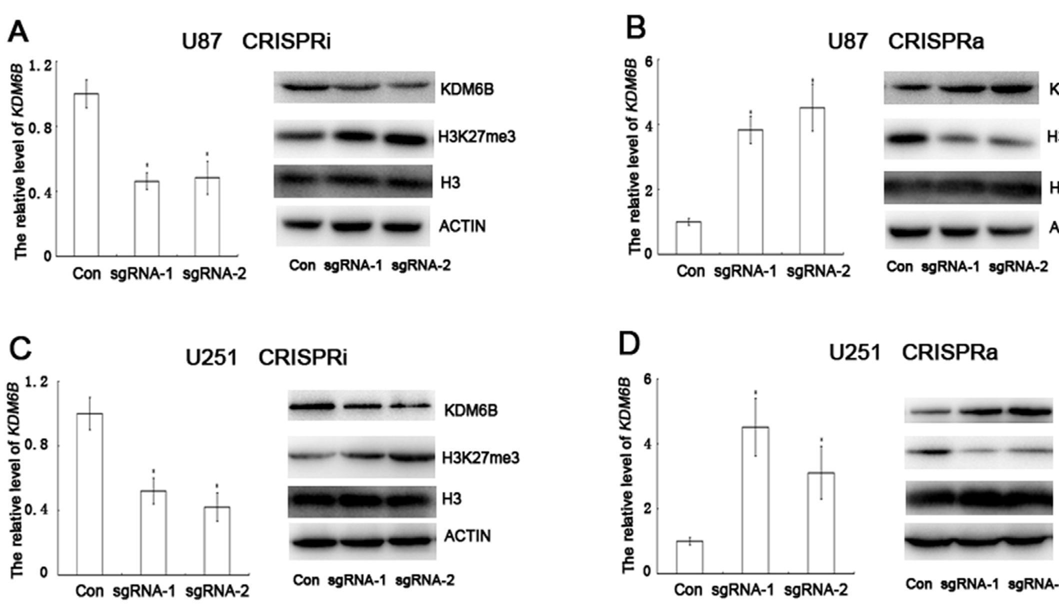

Fig. 1. The KDM6B expression and H3K27me3 content were regulated by CRISPRi or CRISPRa method. (A) U87 cells were transfected with CRISPRi plasmid carrying CRISPRi-sgRNA-1/2 or control sgRNA, and KDM6B mRNA and protein were determined by qPCR or western blotting. (B) U87 cells were transfected with CRISPRa plasmid carrying CRISPRa-sgRNA-1/2 or control sgRNA, and KDM6B mRNA and protein were determined by qPCR or western blotting. (C) U251 cells were performed CRISPRi method. (D) U251 cells were performed CRISPRa method. Values are the mean of triplicate samples from a representative experiment. *, $P < 0.05$.

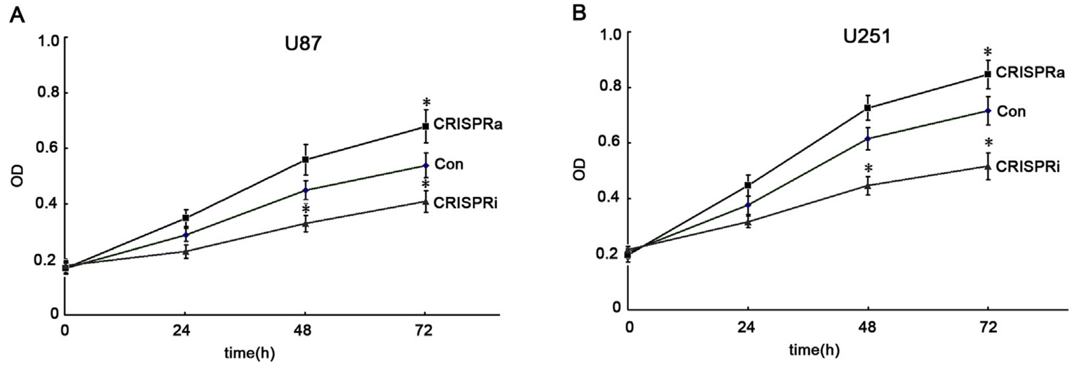

Fig. 2. KDM6B promotes cell proliferation of glioma cells. U87 and U251 cells were performed CRISPRi assay(sgRNA-1) and CRISPRa assay(sgRNA-1). Then, CCK8 assay was performed to investigate the role of KDM6B on cell proliferation. (A) The growth curve of U87 cells. (B) The growth curve of U251 cells. Values are the mean of triplicate samples from a representative experiment. *, $P < 0.05$.

mRNA levels of *SNAI1* in U87 and U251 cells ($P < 0.05$, Fig. 5A and B). Western blot analyses further showed that KDM6B regulated the protein contents of SNAI1 in both glioma cells (Fig. 5C and D). All these results demonstrated that KDM6B maybe affect the epithelial-mesenchymal transition (EMT) of glioma cells.

3.7. KDM6B regulates the H3K27me3 status in promoter of SNAI1

In order to investigate the potentially regulatory mechanism of KDM6B on *SNAI1*, the ChIP assays were performed using KDM6B or H3K27me3 antibody in the *SNAI1* promoter region. The data showed that the contents of KDM6B were significantly reduced and the levels of H3K27me3 were significantly increased on the *SNAI1* promoter in KDM6B knockdown cells compared with control cells ($P < 0.05$,

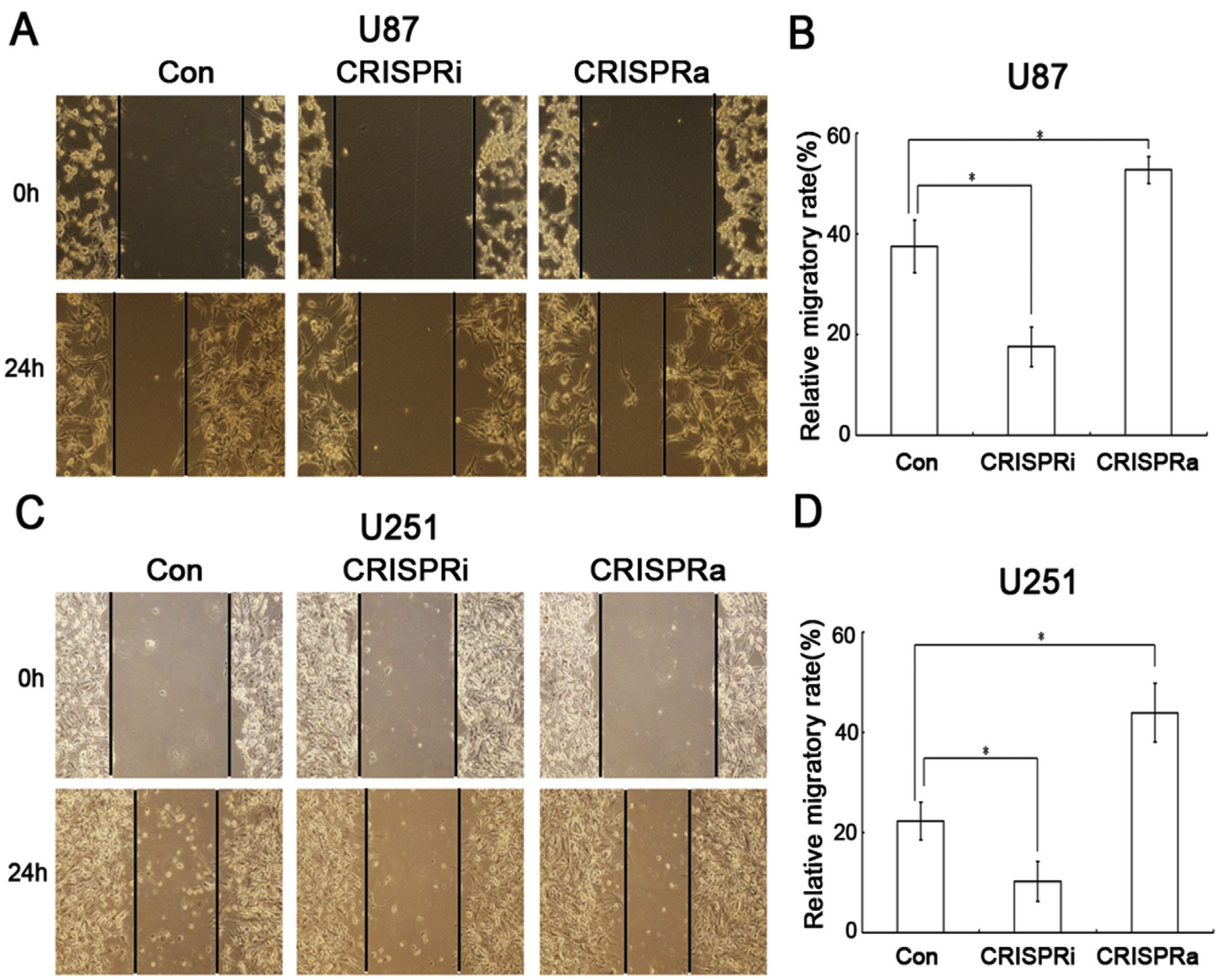

Fig. 3. KDM6B promotes cell migration of glioma cells. U87 and U251 cells were performed CRISPRi assay(sgRNA-1) and CRISPRa assay(sgRNA-1). Then, cell scratch assay was performed to investigate the role of KDM6B in cell migration. (A) The migration of U87 cells. (B) Statistical analysis of U87 cell migration. (C) The migration of U251 cells. (D) Statistical analysis of U251 cell migration. The relative migratory rate can be calculated as followed. The average scratch width at 0 h minuses the width at 24 h, and then divides by the width at 0 h. Values are the mean of triplicate samples from a representative experiment. *, $P < 0.05$.

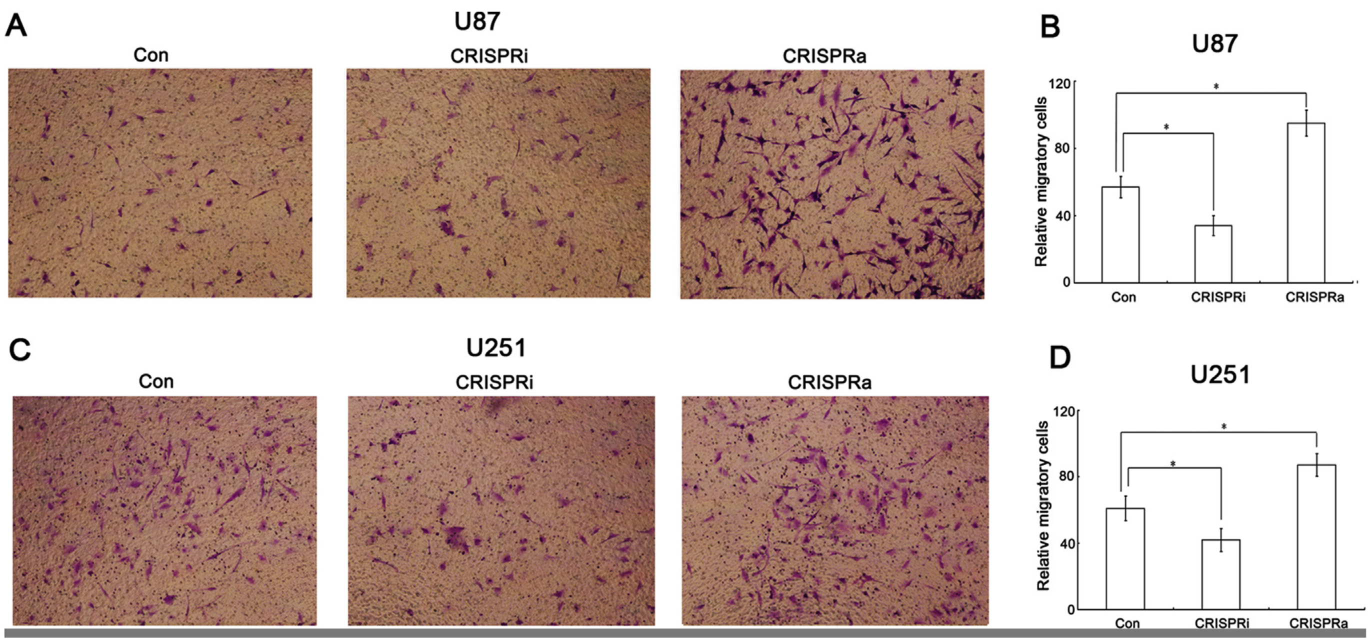

Fig. 4. KDM6B promotes cell invasion of glioma cells U87 and U251. U87 and U251 cells were performed CRISPRi assay(sgRNA-1) and CRISPRa assay(sgRNA-1). Then, a transwell assay was performed to investigate the role of KDM6B in cell invasion. (A) The invasion of U87 cells. (B) Statistical analysis of U87 cell invasion. (C) The invasion of U251 cells. (D) Statistical analysis of U251 cell invasion. Values are the mean of triplicate samples from a representative experiment. *, $P < 0.05$.

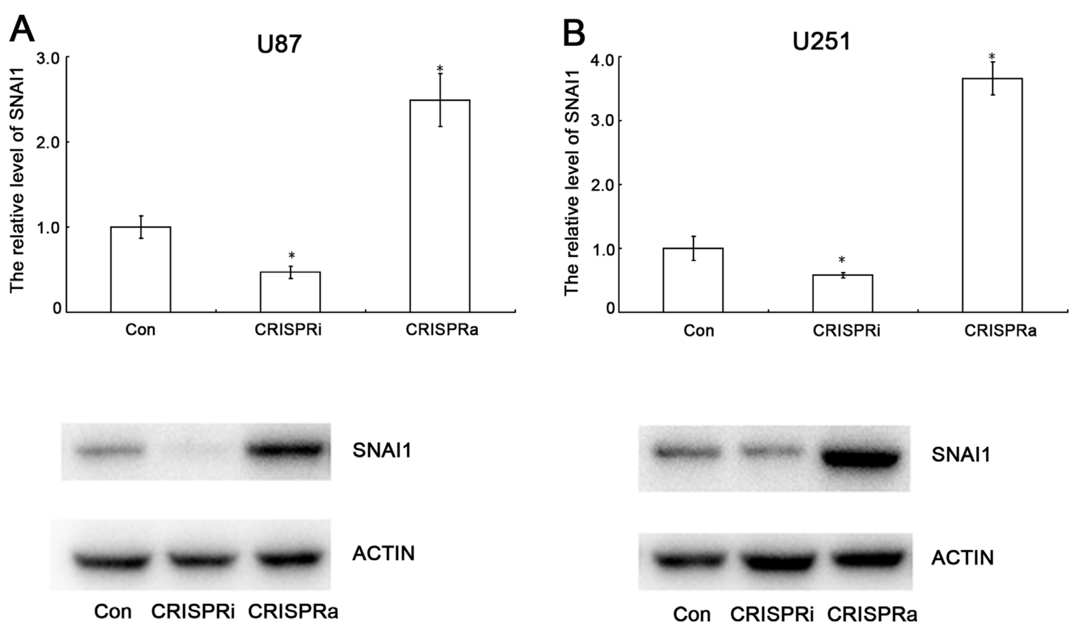

Fig. 5. KDM6B increases SNAI1 expression. U87 and U251 cells were performed CRISPRi assay(sgRNA-1) and CRISPRa assay(sgRNA-1). Then, qPCR and western blotting analyses were performed to investigate the effect of KDM6B on EMT marker SNAI1. (A) The qPCR results (top) and western results (bottom) of U87 cells. (B) The qPCR results (top) and western results (bottom) of U251 cells. Values are the mean of triplicate samples from a representative experiment. *, $P < 0.05$.

Fig. 6A and B). On the contrary, the contents of KDM6B were significantly increased and the levels of H3K27me3 were significantly reduced on the *SNAI1* promoter in KDM6B overexpressed cells compared with control cells ($P < 0.05$, Fig. 6A and B). Thus, it is proved that KDM6B can regulate *SNAI1* expression by direct binding with *SNAI1* promoter and then reducing the H3K27me3 levels.

4. Discussion

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein (CRISPR-Cas9) is a prokaryotic immune system and had been an important tool for gene/genome editing (Jinek et al., 2012; Doudna and Charpentier, 2014). CRISPRi technique was first reported based on CRISPR-Cas9 in 2013 (Qi et al., 2013). The basic principle of CRISPRi is fusion of endonuclease-deficient dCas9 (holding recognition function) with a repressor domain (such as KRAB), which can specifically repress transcription of target gene (Gilbert et al., 2013). CRISPRa can be activate transcription of target gene by fusing a transcriptional activator (such as VP16) to dCas9(Gilbert et al., 2014). Both CRISPRi and CRISPRa have been important tools for gene regulation (La Russa and Qi, 2015), which is essential to investigate gene function and mechanism. CRISPR-Cas9 system can be completed in cells by plasmid transfection (Ran et al., 2014). In the study, we achieved knockdown and overexpressed effect of KDM6B with CRISPRi and

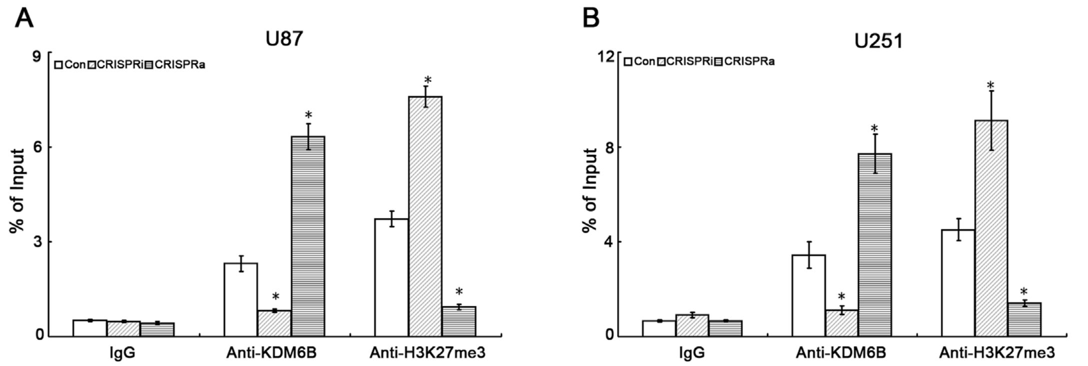

Fig. 6. KDM6B can bind and then decrease H3K27me3 levels in promoter region of *SNAI1*. U87 and U251 cells were performed CRISPRi assay(sgRNA-1) and CRISPRa assay(sgRNA-1). Then, ChIP assays were performed to investigate the effect of KDM6B on *SNAI1*. (A) U87 cells (B)U251 cells. Values are the mean of triplicate samples from a representative experiment. *, $P < 0.05$.

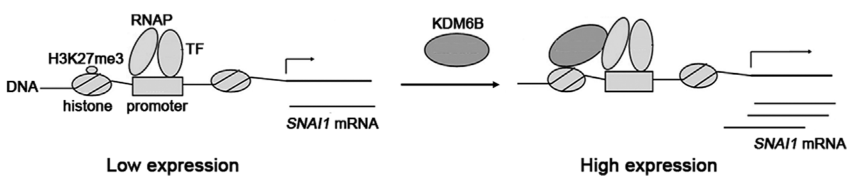

Fig. 7. The model of KDM6B promoting *SNAI1* expression. Lack of KDM6B, RNA polymerase (RNAP) and transcription factor (TP) can only increase moderately expression of *SNAI1*. The recruitment of KDM6B can achieve high *SNAI1* expression by catalyzing H3K27me3 demethylation and activating RNAP activity.

CRISPRa by plasmid transfection, respectively. The negative correlation between KDM6B content and H3K27me3 level also proved that both technologies are feasible for studying the function of KDM6B.

Previous studies have shown that KDM6B is associated with a variety of cancers (Perrigue et al., 2016), especially in metastasis process. In melanoma, KDM6B expression was positively correlated with malignant grades, and KDM6B can promote melanoma metastasis (Park et al., 2016). In hepatocellular carcinoma, levels of KDM6B were correlated inversely with patient survival, and the overexpression of KDM6B induced invasive migration of hepatocellular carcinoma cells (Tang et al., 2016). Additionally, KDM6B expression was also higher in metastatic prostate cancer and renal cell carcinoma (Xiang et al., 2007; Li et al., 2015). Both U87 and U251 are widely used as human glioblastoma cells (Li et al., 2017; Qian et al., 2017), although U87 is a cell line whose origin remains unclear (Allen et al., 2016). Our results indicated that KDM6B promote cell migration and invasion of U87 and U251, two typical cell behaviors of malignant glioma. It further confirmed that KDM6B is also an important promoting factor for glioma metastasis.

Epithelial-mesenchymal transition (EMT) is a basic process of morphogenesis and organ development, which also involved in cancer progression and metastasis (Ribatti, 2017). Many studies have demonstrated that KDM6B can promote EMT and upregulate EMT related genes' expression, such as *SNAI1* and *SLUG* (Ramadoss et al., 2012; Tang et al., 2016; Li et al., 2015). Our data further proved the conclusion in glioma cells. The knockdown and overexpression assays obviously suggested that *SNAI1* expression is dependent on KDM6B. Previous researches have revealed that SNAI1 is up-regulated in glioma tissues (Dong et al., 2014), and involved in the proliferation and migration of glioblastoma cells (Han et al., 2011). Research also showed that SNAI1 plays an oncogenic role in glioblastoma by promoting EMT (Myung et al., 2014). Therefore, our results further suggested that increasing SNAI1 expression may be regarded as one of the reasons for promoting glioma cell migration.

KDM6B is an important transcription activator. KDM6B can promote RNA polymerase II progression and transcriptional elongation (Chen et al., 2012; Estarás et al., 2013), by which KDM6B facilitates transcriptional activation. As a histone demethylase, KDM6B also catalyzes the demethylation of repressive mark H3K27me3, so further upregulates gene expression (Kruidenier et al., 2013; Ntziachristos et al., 2014). ChIP data showed that KDM6B not only accelerates transcription by binding the promoter region of *SNAI1*, but also removes inhibition by reducing H3K27me3 levels. These data further confirmed KDM6B has regulatory role on *SNAI1* expression. Collectively, our study reveals a regulatory mechanism by KDM6B to increase *SNAI1* expression (Fig. 7), which may be one of the mechanisms of KDM6B involvement in glioma metastasis.

Previous researches have showed that deregulation of KDM6B may contribute to gliomagenesis (Ene CI et al., 2012; Perrigue et al., 2015). It also indicated that KDM6B is important for the maintenance of self-renewal of glioblastoma stem cells (Sherry-Lynes et al., 2017). So, inhibition of KDM6B can be considered as a therapeutic strategy for glioma (Hashizume et al., 2014; Sui et al., 2017). It means that this study is of great significance for glioma treatment, especially malignant glioma.

5. Conclusion

In this study, we firstly performed CRISPRi/CRISPRa assay to achieve the expression regulation of histone demethylase KDM6B. Then, we proved that the promoting role of KDM6B in glioma cell proliferation, migration and invasion. Finally, we demonstrated that KDM6B can promote *SNAI1* expression by reducing H3K27me3 levels. These results are helpful in understanding the metastatic mechanism of glioma, and also provide an important target for glioma treatment.

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgments

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References

Agger, K., Cloos, P.A., Christensen, J., Pasini, D., Rose, S., Rappsilber, J., Issaeva, I., Canaani, E., Salcini, A.E., Helin, K., 2007. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 449, 731–734.

Allen, M., Bjerke, M., Edlund, H., Nelander, S., Westermark, B., 2016. Origin of the U87MG glioma cell line: good news and bad news. Sci. Transl. Med. 8, 354re3.
Anderton, J.A., Bose, S., Vockerodt, M., Vrzalikova, K., Wei, W., Kuo, M., Helin, K., Christensen, J., Rowe, M., Murray, P.G., Woodman, C.B., 2011. The H3K27me3 demethylase, KDM6B, is induced by Epstein-Barr virus and over-expressed in Hodgkin's Lymphoma. Oncogene 30, 2037–2043.
Arcipowski, K.M., Martinez, C.A., Ntziachristos, P., 2016. Histone demethylases in physiology and cancer: a tale of two enzymes, JMJD3 and UTX. Curr. Opin. Genet. Dev. 36, 59–67.
Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. Cell Res. 21, 381–395.
Burchfield, J.S., Li, Q., Wang, H.Y., Wang, R.F., 2015. JMJD3 as an epigenetic regulator in development and disease. Int. J. Biochem. Cell Biol. 67, 148–157.
Chen, S., Ma, J., Wu, F., Xiong, L.J., Ma, H., Xu, W., Lv, R., Li, X., Villen, J., Gygi, S.P., Liu, X.S., Shi, Y., 2012. The histone H3 Lys 27 demethylase JMJD3 regulates gene expression by impacting transcriptional elongation. Genes Dev. 26, 1364–1375.
Dong, Q., Cai, N., Tao, T., Zhang, R., Yan, W., Li, R., Zhang, J., Luo, H., Shi, Y., Luan, W., Zhang, Y., You, Y., Wang, Y., Liu, N., 2014. An axis involving SNAI1, microRNA-128 and SP1 modulates glioma progression. PLoS One 9 e98651.
Doudna, J.A., Charpentier, E., 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 1258096.
Ene, C.I., Edwards, L., Riddick, G., Baysan, M., Woolard, K., Kotliarova, S., Lai, C., Belova, G., Cam, M., Walling, J., Zhou, M., Stevenson, H., Kim, H.S., Killian, K., Veenstra, T., Bailey, R., Song, H., Zhang, W., Fine, H.A., 2012. Histone demethylase Jumonji D3 (JMJD3) as a tumor suppressor by regulating p53 protein nuclear stabilization. PLoS One 7 e51407.
Estarás, C., Fueyo, R., Akizu, N., Beltrán, S., Martínez-Balbás, M.A., 2013. RNA polymerase II progression through H3K27me3-enriched gene bodies requires JMJD3 histone demethylase. Mol. Biol. Cell 24, 351–360.
Ge, C., Ye, J., Weber, C., Sun, W., Zhang, H., Zhou, Y., Cai, C., Qian, G., Capel, B., 2018. The histone demethylase KDM6B regulates temperature-dependent sex determination in a turtle species. Science 360, 645–648.
Gilbert, L.A., Horlbeck, M.A., Adamson, B., Villalta, J.E., Chen, Y., Whitehead, E.H., Guimaraes, C., Panning, B., Ploegh, H.L., Bassik, M.C., Qi, L.S., Kampmann, M., Weissman, J.S., 2014. Genome-scale CRISPR-mediated control of gene repression and activation. Cell 159, 647–661.
Gilbert, L.A., Larson, M.H., Morsut, L., Liu, Z., Brar, G.A., Torres, S.E., Stern-Ginossar, N., Brandman, O., Whitehead, E.H., Doudna, J.A., Lim, W.A., Weissman, J.S., Qi, L.S., 2013. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell 154, 442–451.
Greer, E.L., Shi, Y., 2012. Histone methylation: a dynamic mark in health, disease and inheritance. Nat. Rev. Genet. 13, 343–357.
Han, S.P., Kim, J.H., Han, M.E., Sim, H.E., Kim, K.S., Yoon, S., Baek, S.Y., Kim, B.S., Oh, S.O., 2011. SNAI1 is involved in the proliferation and migration of glioblastoma cells. Cell. Mol. Neurobiol. 31, 489–496.
Hashizume, R., Andor, N., Ihara, Y., Lerner, R., Gan, H., Chen, X., Fang, D., Huang, X., Tom, M.W., Ngo, V., Solomon, D., Mueller, S., Paris, P.L., Zhang, Z., Petritsch, C., Gupta, N., Waldman, T.A., James, C.D., 2014. Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. Nat. Med. 20, 1394–1396.
Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E., 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816–821.
Kooistra, S.M., Helin, K., 2012. Molecular mechanisms and potential functions of histone demethylases. Nat. Rev. Mol. Cell Biol. 13, 297–311.
Kruidenier, L., Chung, C.W., Cheng, Z., Liddle, J., Che, K., Joberty, G., Bantscheff, M., Bountra, C., Bridges, A., Diallo, H., Eberhard, D., Hutchinson, S., Jones, E., Katso, R., Leveridge, M., Mander, P.K., Mosley, J., Ramirez-Molina, C., Rowland, P., Schofield, C.J., Sheppard, R.J., Smith, J.E., Swales, C., Tanner, R., Thomas, P., Tumber, A., Drewes, G., Oppermann, U., Patel, D.J., Lee, K., Wilson, D.M., 2012. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. Nature 488, 404–408.
Lan, F., Bayliss, P.E., Rinn, J.L., Whetstine, J.R., Wang, J.K., Chen, S., Iwase, S., Alpatov, R., Issaeva, I., Canaani, E., Roberts, T.M., Chang, H.Y., Shi, Y., 2007. A histone H3 lysine 27 demethylase regulates animal posterior development. Nature 449, 689–694.
La Russa, M.F., Qi, L.S., 2015. The new state of the art: Cas9 for gene activation and repression. Mol. Cell Biol. 35, 3800–3809.
Li, B., Carey, M., Workman, J.L., 2007. The role of chromatin during transcription. Cell 128, 707–719.
Li, Q., Hou, L., Ding, G., Li, Y., Wang, J., Qian, B., Sun, J., Wang, Q., 2015. KDM6B induces epithelial-mesenchymal transition and enhances clear cell renal cell carcinoma metastasis through the activation of SLUG. Int. J. Clin. Exp. Pathol. 8, 6334–6344.
Li, X., Yu, W., Qian, X., Xia, Y., Zheng, Y., Lee, J.H., Li, W., Lyu, J., Rao, G., Zhang, X., Qian, C.N., Rozen, S.G., Jiang, T., Lu, Z., 2017. Nucleus-translocated ACSS2 promotes gene transcription for lysosomal biogenesis and autophagy. Mol. Cell. 66, 684–697 e9.
Myung, J.K., Choi, S.A., Kim, S.K., Wang, K.C., Park, S.H., 2014. Snail plays an oncogenic role in glioblastoma by promoting epithelial mesenchymal transition. Int. J. Clin. Exp. Pathol. 7, 1977–1987.
Ntziachristos, P., Tsirigos, A., Welstead, G.G., Trimarchi, T., Bakogianni, S., Xu, L., Loizou, E., Holmfeldt, L., Strikoudis, A., King, B., Mullenders, J., Becksfort, J., Nedjic, J., Paietta, E., Tallman, M.S., Rowe, J.M., Tonon, G., Satoh, T., Kruidenier, L., Prinjha, R., Akira, S., Van Vlierberghe, P., Ferrando, A.A., Jaenisch, R., Mullighan, C.G., Aifantis, I., 2014. Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia. Nature 514, 513–517.
Park, W.Y., Hong, B.J., Lee, J., Choi, C., Kim, M.Y., 2016. H3K27 Demethylase JMJD3 employs the NF-κB and BMP signaling pathways to modulate the tumor microenvironment and promote melanoma progression and metastasis. Cancer Res. 76, 161–170.
Perrigue, P.M., Najbauer, J., Barciszewski, J., 2016. Histone demethylase JMJD3 at the intersection of cellular senescence and cancer. Biochim. Biophys. Acta 1865, 237–244.
Perrigue, P.M., Silva, M.E., Warden, C.D., Feng, N.L., Reid, M.A., Mota, D.J., Joseph, L.P., Tian, Y.I., Glackin, C.A., Gutova, M., Najbauer, J., Aboody, K.S., Barish, M.E., 2015. The histone demethylase jumonji coordinates cellular senescence including secretion of neural stem cell-attracting cytokines. Mol. Canc. Res. 13, 636–650.
Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., Lim, W.A., 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152, 1173–1183.
Qian, X., Li, X., Cai, Q., Zhang, C., Yu, Q., Jiang, Y., Lee, J.H., Hawke, D., Wang, Y., Xia, Y., Zheng, Y., Jiang, B.H., Liu, D.X., Jiang, T., Lu, Z., 2017. Phosphoglycerate kinase 1 phosphorylates Beclin1 to induce autophagy. Mol. Cell. 65, 917–931 e6.
Ramadoss, S., Chen, X., Wang, C.Y., 2012. Histone demethylase KDM6B promotes epithelial-mesenchymal transition. J. Biol. Chem. 287, 44508–44517.
Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8, 2281–2308.
Ribatti, D., 2017. Epithelial-mesenchymal transition in morphogenesis, cancer progression and angiogenesis. Exp. Cell Res. 353, 1–5.
Shen, Y., Guo, X., Wang, Y., Qiu, W., Chang, Y., Zhang, A., Duan, X., 2012. Expression and significance of histone H3K27 demethylases in renal cell carcinoma. BMC Canc. 12, 470.
Sherry-Lynes, M.M., Sengupta, S., Kulkarni, S., Cochran, B.H., 2017. Regulation of the JMJD3 (KDM6B) histone demethylase in glioblastoma stem cells by STAT3. PLoS One 12 e0174775.
Sui, A., Xu, Y., Li, Y., Hu, Q., Wang, Z., Zhang, H., Yang, J., Guo, X., Zhao, W., 2017. The pharmacological role of histone demethylase JMJD3 inhibitor GSK-J4 on glioma cells. Oncotarget 8, 68591–68598.
Tang, B., Qi, G., Tang, F., Yuan, S., Wang, Z., Liang, X., Li, B., Yu, S., Liu, J., Huang, Q., Wei, Y., Zhai, R., Lei, B., Yu, H., Tomlinson, S., He, S., 2016. Aberrant JMJD3 expression upregulates slug to promote migration, invasion, and stem cell-like behaviors in hepatocellular carcinoma. Cancer Res. 76, 6520–6532.
Xiang, Y., Zhu, Z., Han, G., Lin, H., Xu, L., Chen, C.D., 2007. JMJD3 is a histone H3K27 demethylase. Cell Res. 17, 850–857.